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Osteosarcoma, personalized medicine, and tissue engineering; an overview of overlapping fields of research

Azam Bozorgi *, Leila Sabouri **

Department of Tissue Engineering and Regenerative Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

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ABSTRACT

Introduction: Osteosarcoma is a common bone malignancy in patients of all ages. Surgical and chemotherapy interventions fail to shrink tumor growth and metastasis. The development of efficient patient-specific therapeutic strategies for osteosarcoma is of great interest in tissue engineering and personalized medicine. The present manuscript aimed to review the advancements in tissue engineering and personalized medicine strategies to overcome osteosarcoma and the relevant biological aspects as well as the current tumor models *in vitro* and *in vivo*.

Results: Tissue engineering and personalized medicine contribute to gene/cell engineering and cell-based therapies specific to genomic and proteomic profiles of individual patients to improve the current treatment options. Also, tissue engineering scaffolds provide physical support to missing bones, could trap cancer cells and deliver immune cells. Taken together, these strategies suppress tumor growth, angiogenic potential, and the subsequent metastasis as well as elicit desirable immune responses against tumor mass.

Discussion: Advanced and high-throughput gene and protein identification technologies have facilitated the recognition of genomic and proteomic profiles of patients to design and develop patient-specific treatments. The pre-clinical studies showed promising outcomes to inhibit tumor growth and invasion but controversial results compared to clinical investigations make the importance of more clinical reports inevitable. The experimental tumor models assist the evolution of effective treatments by understanding the mechanisms of tumor progression.

Conclusion: Tissue engineering and personalized medicine strategies seem encouraging alternatives to conventional therapies against osteosarcoma. Modeling the tumor microenvironment coupled with pre-clinical results give new intelligence into the translation of strategies into the clinic.

Introduction

Osteosarcoma is the most widespread bone primary malignancy that involves children, youth, and adolescents, and metastasis is diagnosed in 20% of patients [1]. Common therapeutic strategies to treat osteosarcoma account for surgical resection followed by chemotherapy regimens to prevent tumor metastasis [2]. However, chemotherapy-induced toxicities are still the main challenge to the applied chemotherapy treatments [3]. It has been demonstrated that osteosarcoma emergence and progress are associated with genetic variations. The genetic variations and instabilities make the molecular mechanisms of osteosarcoma pathogenesis difficult to understand [4]. These challenges are of great interest to design and carry out patient-specialized investigations. Personalized medicine is an appearing field of medicine regarding the genetic and molecular aspects of diseases like cancer and enables researchers to make decisions to predict, prevent, diagnose, and treat diseases [5]. Next-generation gene sequencing, morphogenomic (the combination of morphology and genomics), and morphoproteomic (the combination of morphology and proteomics) assays are efficient techniques to facilitate the evolution of personalized medicine-based targeted therapies [6]. Tissue engineering and regenerative medicine (TERM) is an interdisciplinary field of medicine, biomaterials, and cell biology contributing to the design and employment of strategies to restore the structure and function of damaged tissues [7]. The field of TERM introduces both *in vitro* and *in vivo* models and therapeutic strategies to surpass the current knowledge of osteosarcoma biology and

* Corresponding author.
** Corresponding author.
E-mail addresses: abozorgi1991@gmail.com (A. Bozorgi), sabourileila@yahoo.com (L. Sabouri).

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treatment efficacy. Herein, the overlapping areas of personalized medicine and tissue engineering were reviewed as well as the application of tissue engineering in personalized medicine in various directions were discussed.

Osteosarcoma pharmacogenomics; definition and data sources

The concept of pharmacogenomics results from the combination of two words 'pharmacology' and 'genomics.' It is a part of precision medicine and is defined as the use of genome-related technologies to understand how genetic composition influences the drug efficacy and toxicity [8]. The genomic-based drug administration is noteworthy because the drug response under the same therapy condition (drug type, dose, and treatment duration) differs among patients. Large-scale genomic and proteomic databases give valuable data on the whole genome, epigenome, and phenotype to predict the patient response to drugs and relevant activities.

The use of gene sequencing, transcriptional, and computational (in silico) methodologies in preclinical and clinical studies are beneficial to develop and translate personalized drugs into the clinic [9-12]. Moreover, different theories are studied, offer organized roadmaps for designing projects, and eventually, novel drugs or therapeutic options are achieved. Comprehensively, pharmacogenomics research based on computer studies and database exploration is the currently introduced approach to generate novel therapies in precision medicine [11, 12].

It has been revealed that detected biomarkers in patients suffering from osteosarcoma could serve as promising markers in osteosarcoma pharmacogenomics. Studies on ATP-binding cassette transporter 1 (ABCB1), dihydrofolate reductase (DHFR), solute carrier family 19 member 1 (SLC19A1), human epidermal growth factor receptor 2 (HER2) [8], circulating tumor suppressor microRNAs (miR326, miR125b, miR133b, miR206, miR152, miR95–3p, miR34b, miR195, miR223, miR497), oncogenic microRNAs (miR-17, miR21, miR-24, miR-25–3p, miR29a/b/c, miR143, miR196a/b, miR199a-3p, miR-221, miR236,), and long non-coding RNAs (TUG1, 91H, UCA1, ATB, MALAT-1) would be effective to predict tumor prognosis, therapy resistance, and survival [13].

Cell-based personalized therapeutic approaches against osteosarcoma

The tissue engineering (TE) concept relies on the use of biomaterials, cells, and biomolecules, alone or in combination, to provide specific approaches to repair and regenerate damaged tissues or organs [14]. TE strategies are coupled with personalized medicine through cell therapy, cell engineering, and genetic manipulation procedures (Fig. 1). Cell-based strategies serve as the first aim of personalized medicine employed for both somatic and cancer cells. Differentiation therapy (inducing cancer cells into fully differentiated cell lineages) is beneficial to overcome osteosarcoma therapy resistance. Fasudil treatment induced the adipogenic differentiation of highly resistant osteosarcoma cells by remodeling the cytoskeleton arrangement and the blockage of the tumor growth and resistance [15]. Trabectedin prompted the terminal differentiation of osteosarcoma cells into osteoblasts characterized by increased expression of RUNX2, reduced tumorigenicity, and increased population of CD8 T lymphocytes [16].

Genetic editing methods discuss two main issues of selecting appropriate gene targets and designing efficient gene delivery systems. VEGFA gene editing was conducted using a tumor-targeted lipopolymer delivery system carrying the CRISPR/Cas9 plasmids. The inhibition of the VEGFA gene led to reduced angiogenesis and tumor growth as well as suppressed the lung metastasis capacity of osteosarcoma cells [17]. GIT1, a target to osteosarcoma treatment recently introduced by Zhang et al. attributed to the angiogenesis and invasive potential of osteosarcoma cells via activating hypoxia-inducible factor1α (HIF1α) and extracellular signal-regulated kinase (ERK1/2) pathways respectively [18]. The knockdown of PTBP1, an RNA-binding protein gives rise to higher levels of copper transporter 1 protein SLC31A1, responsible for drug influx and cisplatin sensitivity [19]. ALDH1B1 knockdown was associated with inhibited *in vitro* growth, migration, invasion, and induced cell cycle arrest at the G1 phase of osteosarcoma cells and repressed xenograft tumor growth [20]. siRNA nanocarriers of chitosan-folic acid efficiently transferred the astrocyte elevated gene-1 (AEG-1) siRNA into the osteosarcoma cells followed by modulating matrix metalloproteinases 2/9 (MMP-2/9) and diminished tumor growth and metastasis [21].

Adoptive cell therapy (ACT), is a strategy to strengthen the immune cell activities by isolating autologous immune cells, making genetic manipulations, and subsequent reinfusion of genetically modified cells into the patient's body to reach immune responses of interest [22]. A broad range of cell-based therapeutic studies focus on dendritic cells, antigen-presenting members of the immune system. Dendritic cell vaccines provide a remarkable antigen-specific immune response and anti-tumor effect against preclinical osteosarcoma models identified by enhanced cytotoxic T lymphocytes [23] and abolished regulatory T cells [24]. Invariant natural killer cells (iNK), a subpopulation of immune

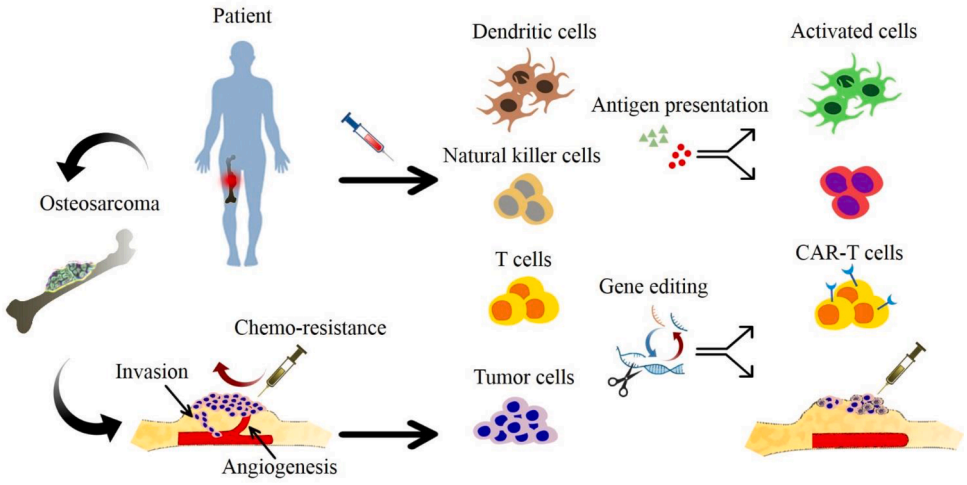

Fig. 1. Tissue engineering/personalized medicine contribution to advance anti-tumor strategies. Patient-specific cell therapies and genetic manipulation endow improved outcomes including tumor repression, inhibited invasion and angiogenesis, active immune responses, and higher sensitivity to current therapeutic regimens.

cells with bifunctional properties of T-cells and NK cells specifically target osteosarcoma cells through CD1d- dependent cytotoxicity and intensify the chemotherapy-induced cell death [25].

Chimeric antigen receptor-engineered T-lymphocytes (CAR T-cells) are well-known engineered cell populations with two main manipulated entities of specific antibody recognition domain and T cell receptor (TCR) to recognize certain antigens followed by activating response signals from TCR [26]. Genetically engineered oncolytic viruses are used as immune response eliciting and cancer cell death mediators in a variety of cancers include osteosarcoma [27]. Clinical CAR T-cell therapy against HER-2 positive osteosarcoma was found safe but the results showed that the CAR T-cell population failed to expand following the infusion [28]. In addition to immune cells, cancer cells can be genetically modified to express specific antigens that prevent them to escape and hide. These solutions could be accomplished by getting access to the genomic and proteomic profiles of the patient [29].

Tissue engineering strategies to overcome osteosarcoma progression

Generally, tissue engineering strategies act to regress the tumor growth as well as heal the tumor site in three directions. First, TE scaffolds could are considered as defect filler constructs; When the osteosarcoma tumor is removed from bone tissue, large defects remain in the tumor site. Herein, tissue-engineered products provide physical support to and fill the gap of missing bone [30, 31]. Given extensive studies conducted, several tissue-engineered products received the necessary licenses and have been commercialized for use in the clinic [32-35]. An example of personalized tissue-engineered products is 3D-printed titanium implants with improved biomechanical properties that ameliorate the patient's clinical outcome [36].

Second, TE scaffolds actively repress tumor growth and metastasis in several ways. Experimental investigations indicated that scaffolds implanted in the vicinity of the primary tumor strongly reduced the rate of metastasis from the primary tumor to tissues such as bone, lung, etc. A study on breast cancer biology evidenced that biomaterial scaffolds could modulate the tumor microenvironment, thereby immune cell secretions and phenotypes shift in favor of tumor suppression [37]. Microporous polycaprolactone scaffolds could trap breast cancer cells and prevent them from metastasizing to the tissues resulting in impaired tumor recurrence and prolonged survival [38]. Also, biomaterials increase the metabolism and migration of the host immune cells making them new candidates as adjuvants or therapeutic vaccines [39, 40]. In the third mechanism of anti-tumor action, scaffolds could carry a population of antigen-presenting cells and immune system modulators to intensify the immune system against cancerous tissue [38, 41].

Small biomolecules play a considerable role in restaining osteosarcoma growth and invasion. TE scaffolds delivered vitamin C, not only diminished the proliferation of osteosarcoma cells but also stimulated osteogenic progression [42]. *All-trans* retinoic acid (ATRA) prohibited the polarization of tumor-associated macrophages (TAMs) into M2 phenotype via inhibiting the IL-13/14 production and induced a negative effect on cancer cell migration [43]. Dihydroxycoumarins displayed a dual direct and indirect function of G1 cell cycle arrest and suppression of M2 macrophage differentiation respectively [44].

The next generation of TE products with progressed efficiency in personalized medicine is necessary to develop according to the genotypic and phenotypic profiles of patients. This leads to a variety of products in different groups prepared based on age, sex, race, and specialized structure and function of interest for each person.

Personalized osteosarcoma models; *in vitro* and *in vivo* evidence

In vitro models

2D culture: Monoculture models (monolayer, transwell, conditioned medium) are regarded as the first applied models in bone cancer research. Osteosarcoma cultures are usually obtained from both normal and cancerous sources ((Fig. 2). Genetically modified mesenchymal stem cells (MSCs) with retinoblastoma (Rb) knockdown and c-Myc overexpression resulted in osteosarcoma formation associated with enhanced cell growth and sphere formation *in vitro* [45]. It is suggested that the source of MSCs and osteogenic commitment are key factors to succeed in the formation of osteosarcoma models. For example, P53/retinoblastoma deficient bone marrow-derived MSCs undergoing osteogenic commitment exhibit a greater rate of bone sarcomas than undifferentiated MSCs or osteogenic precursors derived from adipose tissue MSCs [46]. Although, some evidence concludes that interfering with *Trp53* and *Rb1* expression successfully induces MSCs, pre-osteoblasts, and fully differentiated osteoblasts into osteosarcoma cells [47]. Some cancer-related genetic disorders provide suitable personalized osteosarcoma models. Li-Fraumeni syndrome (LFS) is an autosomal dominant inherited disease that makes patients susceptible to different types of cancers including osteosarcoma. Induced pluripotent stem cells (iPSCs) from LFS patients that were differentiated into osteoblasts showed impaired expression of P53 and H19 leading to tumor recurrence [48]. Moreover, the increased expression of secreted frizzled-related protein 2 in LFS derived iPSCs promoted osteosarcomagenesis via prompting *FOXM1* and *CYR61*oncogens [49].

3D culture: Despite the extended studies on osteosarcoma biology and treatment, 2D tumor models are unable to depict a comprehensive explanation of the physico/chemical state of the tumor microenvironment, cell-cell, cell-ECM interactions, drug resistance, and tumor heterogeneity [50]. So, the presentation of 3D models that mimic the tumor microenvironment seems inevitable. 3D tumor models are categorized into scaffold-based, cell-based, and mixed models (Fig. 2). In scaffold-based models, synthetic and natural materials such as bone extracellular matrix [51], collagen [52], silk [53], poly-caprolactone (PCL) [54], poly(lactic acid-co-glycolic acid) (PLGA) [55], as solid scaffolds or hydrogels are utilized in bone cancer modeling.

Tumor cell spheroids known as 3D cell-based models could be generated using both static methods including hanging drop [56] and dynamic conditions such as spinner flasks, stirred-tank cultures, and bioreactors [57]. Osteosarcoma spheroids have a stronger drug resistance potential than monolayer cultures attributed to the elevated expression of cathepsin D [58]. The cell/scaffold complexes as newly introduced artificial tumor niches that open new insights into the interaction of osteosarcoma cells with their microenvironment. The combination of adipose-derived mesenchymal stem cells (ADMSCs) and gellan gum (GG)-silk fibroin scaffolds provide a micro-niche with appropriate stiffness to increase the proliferation, spheroid formation, and osteosarcoma- related gene expression of saos-2 cells [59]. Hydrogel microspheres of hyaluronan/gelatin presented a useful platform for bone metastasis of prostate cancer [60].

In vivo models

Patient-derived osteosarcoma xenografts are common models to evaluate the development of bone cancer and the underlying molecular mechanisms. Specimens are obtained from patients suffering from osteosarcoma, subcutaneously transplanted into immunodeficient animals followed by the re-transplantation of newly formed tumors into new hosts. Molecular and histological assessments of implanted tumors aid researchers to uncover key aspects of osteosarcoma formation and progression [61]. The *in vivo* humanized osteosarcoma model was generated from implantable 3D-printed PCL scaffolds in combination with hydrogel embedded human umbilical cord vein endothelial cells and human osteosarcoma cells [54].

Personalized combination therapies against osteosarcoma

The common therapeutic options for patients that suffer from

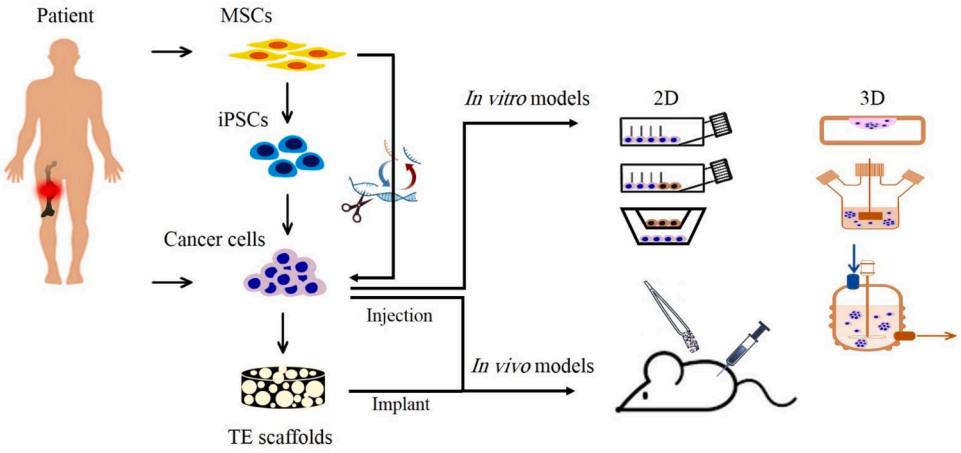

Fig. 2. A schematic view of *in vitro* and *in vivo* osteosarcoma models. Different normal and cancer cell sources are used to model tumor microenvironment. Mesenchymal stem cells (MSCs) are genetically modified into induced pluripotent stem cells (iPSCs) or cancer cells. Cancer cells are used to develop 2D (monolayer, co-culture, transwell) and 3D (hanging drop, spinner flask, bioreactor) *in vitro* models. Cancer cells individually or in combination with tissue engineering (TE) scaffolds are transplanted into laboratory animals to give rise to *in vivo* models.

osteosarcoma are surgical removal and subsequent chemotherapies to suppress the metastatic progression of the tumor. The common chemotherapy regimens include a combination of cisplatin, doxorubicin, and high-dose methotrexate with leucovorin rescue (MAP) [62]. Long-term consequences such as nephrotoxicity and cardiotoxicity followed by MAP administration makes scientists seek new alternatives [3]. Some agents have been presented as neoadjuvant such as high-dose ifosfamide and etoposide(I/E) [63] and interferon alfa-2b [64] but they lack significant therapeutic efficiency.

Personalized chemotherapy regimens enable researchers to understand the mechanisms of genome-related drug metabolism and resistance, osteosarcoma-related gene mutations, and mapping the gene correlations [65]. These findings along with optimized tissue engineering-based drug delivery strategies give new imagination into the design and development of more efficient personalized treatments. A promising strategy against osteosarcoma is encapsulating Muramyl tripeptide phosphatidylethanolamine (MTP-PE) into liposomes to actuate monocytes and macrophages [66]. A combination therapy based on MAP-MTP-PE has been elucidated to upgrade the free survival and overall survival of patients [67]. Immunotherapy strategies based on PD1/PDL1 communication have been found to improve patient survival and diminished metastasis in PDL1 expressing osteosarcoma cells [68].

Endoglin-targeting antibodies conjugated with nigrin-b A chain and cytolysin successfully inhibited the proliferation of Ewing sarcoma cells and repressed the patient-derived tumor growth *in vivo* [69]. Co-administration of eribulin and temozolomide to patient-derived orthotopic xenograft (PDOX) model suppressed the tumor growth and resistance to a variety of agents such as doxorubicin, sunitinib, and pazopanib [70]. A combination of PARP inhibitor, olaparib, and doxorubicin inhibited the osteosarcoma cell growth and induced apoptosis [71]. Co-treatment of therapeutic agents with cells such as oncolytic viruses and cisplatin [72], dendritic cells with antibodies of transforming growth factor-β (TGF-β) [73], and glucocorticoid-induced tumor necrosis factor receptor (GITR) [24] was accompanied by an enhanced rate of autophagy *in vitro* and suppressed lung metastasis *in vivo*.

Discussion and conclusion

Personalized medicine and tissue engineering are two major fields concerning bone cancer research and treatment aim in the development of advanced targeted strategies to compensate for the present therapeutic options and defeat tumor growth and metastasis. Targeted cancer therapies require the identification of genetic alterations of individual patients as well as the effect of genomic variations on the patient response to treatment should be considered. High-throughput technologies like next-generation sequencing enrich the current knowledge to achieve an efficient therapy. Comprehensive preclinical researches and clinical trials are needed to ensure the efficacy and feasibility of currently developed strategies. The common spots in personalized medicine and tissue engineering are gene engineering, cell engineering, and cell-based therapies to suppress cancer cell metabolism, growth, and invasion. Immune cells play a prominent role in the modulation of the tumor microenvironment. Tissue engineering scaffolds not only support the structure of tumor-damaged bone but also prohibit the cancer cell growth and metastasis via trapping cancer cells and regulating tumor niche status as well as deliver immune cells to the tumor site to take favorable immune responses. Small biomolecules own the same effects toward tumor repression. Although the conventional chemotherapy regimens have been combined with immunotherapy agents such as interleukin-2 (IL-2) [74] and IGF-1R antibodies [75], no significant clinical outcome was not found in patients. Clinical studies on dendritic cell-based vaccines did not face the same success as preclinical ones [76]. Therefore, it seems that the controversial results of preclinical and clinical examinations are challenging issues in front of clinical translation of personalized medicine and tissue engineering procedures.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] M.E. Anderson, Update on survival in osteosarcoma, Orthop Clin North Am 47 (1) (2016) 283–292.
[2] A. Luetke, et al., Osteosarcoma treatment - where do we stand? A state of the art review, Cancer Treat Rev 40 (4) (2014) 523–532.
[3] C.L. Schwartz, et al., Intensified chemotherapy with dexrazoxane cardioprotection in newly diagnosed nonmetastatic osteosarcoma: a report from the Children's Oncology Group, Pediatr Blood Cancer 63 (1) (2016) 54–61.
[4] D. Egas-Bejar, et al., Theranostic profiling for actionable aberrations in advanced high risk osteosarcoma with aggressive biology reveals high molecular diversity: the human fingerprint hypothesis, Oncoscience 1 (2) (2014) 167–179.
[5] M. Di Sanzo, et al., Clinical applications of personalized medicine: a new paradigm and challenge, Curr Pharm Biotechnol 18 (3) (2017) 194–203.
[6] R.E. Brown, Morphogenomics and morphoproteomics: a role for anatomic pathology in personalized medicine, Arch Pathol Lab Med 133 (4) (2009) 568–579.
[7] M.R. Borrelli, et al., Tissue engineering and regenerative medicine in craniofacial reconstruction and facial aesthetics, J Craniofac Surg 31 (1) (2020) 15–27.

[8] M. Serra, C.M. Hattinger, The pharmacogenomics of osteosarcoma, Pharmacogenomics J 17 (1) (2017) 11–20.
[9] R. Groisberg, et al., Clinical genomic profiling to identify actionable alterations for investigational therapies in patients with diverse sarcomas, Oncotarget 8 (24) (2017) 39254–39267.
[10] C. Rodríguez-Antona, M. Taron, Pharmacogenomic biomarkers for personalized cancer treatment, J Intern Med 277 (2) (2015) 201–217.
[11] L. Zhang, et al., Three-dimensional (3D) printed scaffold and material selection for bone repair, Acta Biomater 84 (2019) 16–33.
[12] J. Giri, et al., Concepts driving pharmacogenomics implementation into everyday healthcare, Pharmgenomics Pers Med 12 (2019) 305–318.
[13] L. Raimondi, et al., Circulating biomarkers in osteosarcoma: new translational tools for diagnosis and treatment, Oncotarget 8 (59) (2017) 100831–100851.
[14] R. Shi, et al., Current advances for bone regeneration based on tissue engineering strategies, Front Med 13 (2) (2019) 160–188.
[15] N. Takahashi, et al., ROCK inhibition induces terminal adipocyte differentiation and suppresses tumorigenesis in chemoresistant osteosarcoma cells, Cancer Res 79 (12) (2019) 3088–3099.
[16] C. Ratti, et al., Trabectedin overrides osteosarcoma differentiative block and reprograms the tumor immune environment enabling effective combination with immune checkpoint inhibitors, Clin Cancer Res. 23 (17) (2017) 5149–5161.
[17] C. Liang, et al., Tumor cell-targeted delivery of CRISPR/Cas9 by aptamer-functionalized lipopolymer for therapeutic genome editing of VEGFA in osteosarcoma, Biomaterials 147 (2017) 68–85.
[18] Z. Zhang, et al., Inhibiting GIT1 reduces the growth, invasion, and angiogenesis of osteosarcoma, Cancer Manag Res 10 (2018) 6445–6455.
[19] C. Cheng, et al., PTBP1 modulates osteosarcoma chemoresistance to cisplatin by regulating the expression of the copper transporter SLC31A1, J Cell Mol Med 24 (9) (2020) 5274–5289.
[20] X. Wang, et al., Upregulation of ALDH1B1 promotes tumor progression in osteosarcoma, Oncotarget 9 (2) (2018) 2502–2514.
[21] F. Wang, et al., Nanoscale polysaccharide derivative as an AEG-1 siRNA carrier for effective osteosarcoma therapy, Int J Nanomedicine 13 (2018) 857–875.
[22] Z. Wang, et al., Innate immune cells: a potential and promising cell population for treating osteosarcoma, Front Immunol 10 (2019), 1114-1114.
[23] Y.T. He, et al., In vitro generation of cytotoxic T lymphocyte response using dendritic cell immunotherapy in osteosarcoma, Oncol Lett 12 (2) (2016) 1101–1106.
[24] M. Kawano, et al., Dendritic cells combined with anti-GITR antibody produce antitumor effects in osteosarcoma, Oncol Rep 34 (4) (2015) 1995–2001.
[25] S. Fallarini, et al., Invariant NKT cells increase drug-induced osteosarcoma cell death, Br J Pharmacol 167 (7) (2012) 1533–1549.
[26] C. DeRenzo, S. Gottschalk, Genetically modified T-cell therapy for the treatment of osteosarcoma: an update, J Clin Cell Immunol 7 (2) (2016).
[27] C. Geiss, et al., Preclinical testing of an oncolytic parvovirus: standard protoparvovirus H-1PV efficiently induces osteosarcoma cell lysis in Vitro, Viruses 9 (10) (2017) 301.
[28] N. Ahmed, et al., Human epidermal growth factor receptor 2 (HER2) -specific chimeric antigen receptor-modified T cells for the immunotherapy of HER2-positive sarcoma, J Clin Oncol 33 (15) (2015) 1688–1696.
[29] M. Patel, S.M. Kato, R. Kurzrock, *Molecular tumor boards: realizing precision oncology therapy*, Clin Pharmacol Ther 103 (2) (2018) 206–209.
[30] Ž. Perić Kačarević, et al., An introduction to bone tissue engineering, Int J Artif Organs 43 (2) (2020) 69–86.
[31] B.M. Holzapfel, et al., Tissue engineering and regenerative medicine in musculoskeletal oncology, Cancer and Metastasis Reviews 35 (3) (2016) 475–487.
[32] S. Pina, et al., Scaffolding strategies for tissue engineering and regenerative medicine applications, Materials (Basel) (Basel, Switzerland), 12 (11) (2019) 1824.
[33] J.R. Perez, et al., Tissue engineering and cell-based therapies for fractures and bone defects, Front Bioeng Biotechnol 6 (2018), 105-105.
[34] J. Ng, et al., Biomimetic approaches for bone tissue engineering, Tissue Eng Part B Rev 23 (5) (2017) 480–493.
[35] H.D. Kim, et al., Biomimetic materials and fabrication approaches for bone tissue engineering, Adv Healthc Mater 6 (23) (2017), 1700612.
[36] L. Ma, et al., 3D printed personalized titanium plates improve clinical outcome in microwave ablation of bone tumors around the knee, Sci Rep 7 (1) (2017) 7626.
[37] B.A. Aguado, et al., Biomaterial scaffolds as pre-metastatic niche mimics systemically alter the primary tumor and tumor microenvironment, Adv Healthc Mater 7 (10) (2018) e1700903-e1700903.
[38] S.S. Rao, et al., Enhanced survival with implantable scaffolds that capture metastatic breast cancer cells in vivo, Cancer Res 76 (18) (2016) 5209–5218.
[39] L. Gu, D.J. Mooney, Biomaterials and emerging anticancer therapeutics: engineering the microenvironment, Nat Rev Cancer 16 (1) (2016) 56–66.
[40] B.G. De Geest, Engineering the immune system with particles, step-by-step, Mol Immunol 98 (2018) 25–27.
[41] D.G. Leach, S. Young, J.D. Hartgerink, Advances in immunotherapy delivery from implantable and injectable biomaterials, Acta Biomater 88 (2019) 15–31.
[42] S. Bose, N. Sarkar, S. Vahabzadeh, Sustained release of vitamin C from PCL coated TCP induces proliferation and differentiation of osteoblast cells and suppresses osteosarcoma cell growth, Mater Sci Eng C Mater Biol Appl 105 (2019), 110096.
[43] Q. Zhou, et al., All-trans retinoic acid prevents osteosarcoma metastasis by inhibiting m2 polarization of tumor-associated macrophages, Cancer Immunol Res 5 (7) (2017) 547–559.
[44] Y. Kimura, M. Sumiyoshi, Antitumor and antimetastatic actions of dihydroxycoumarins (esculetin or fraxetin) through the inhibition of M2 macrophage differentiation in tumor-associated macrophages and/or G1 arrest in tumor cells, Eur J Pharmacol 746 (2015) 115–125.
[45] J.Y. Wang, et al., Generation of osteosarcomas from a combination of Rb Silencing and c-Myc overexpression in human mesenchymal stem cells, Stem Cells Transl Med 6 (2) (2017) 512–526.
[46] R. Rubio, et al., The differentiation stage of p53-Rb-deficient bone marrow mesenchymal stem cells imposes the phenotype of in vivo sarcoma development, Oncogene 32 (41) (2013) 4970–4980.
[47] T. Quist, et al., The impact of osteoblastic differentiation on osteosarcomagenesis in the mouse, Oncogene 34 (32) (2015) 4278–4284.
[48] D.F. Lee, et al., Modeling familial cancer with induced pluripotent stem cells, Cell 161 (2) (2015) 240–254.
[49] H. Kim, et al., Oncogenic role of SFRP2 in p53-mutant osteosarcoma development via autocrine and paracrine mechanism, Proc Natl Acad Sci U S A 115 (47) (2018) E11128–e11137.
[50] E.L. Fong, et al., Heralding a new paradigm in 3D tumor modeling, Biomaterials 108 (2016) 197–213.
[51] Y. Zhang, Y. Yao, Y. Zhang, *Three*-dimensional bone extracellular matrix model for osteosarcoma, J Vis Exp (146) (2019) e59271.
[52] K.M. Charoen, et al., Embedded multicellular spheroids as a biomimetic 3D cancer model for evaluating drug and drug-device combinations, Biomaterials 35 (7) (2014) 2264–2271.
[53] P.H. Tan, et al., Three-dimensional porous silk tumor constructs in the approximation of in vivo osteosarcoma physiology, Biomaterials 32 (26) (2011) 6131–6137.
[54] F. Wagner, et al., Humanization of bone and bone marrow in an orthotopic site reveals new potential therapeutic targets in osteosarcoma, Biomaterials 171 (2018) 230–246.
[55] A. Komez, et al., A two-compartment bone tumor model to investigate interactions between healthy and tumor cells, Biomed Mater 15 (3) (2020), 035007.
[56] J.M. Kelm, et al., Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types, Biotechnol Bioeng 83 (2) (2003) 173–180.
[57] V.E. Santo, et al., Adaptable stirred-tank culture strategies for large scale production of multicellular spheroid-based tumor cell models, J Biotechnol 221 (2016) 118–129.
[58] K. Arai, et al., Proteomic approach toward molecular backgrounds of drug resistance of osteosarcoma cells in spheroid culture system, Proteomics 13 (15) (2013) 2351–2360.
[59] B. Kundu, et al., Mechanical property of hydrogels and the presence of adipose stem cells in tumor stroma affect spheroid formation in the 3D osteosarcoma model, ACS Appl Mater Interfaces 11 (16) (2019) 14548–14559.
[60] J. Antunes, et al., In-air production of 3D co-culture tumor spheroid hydrogels for expedited drug screening, Acta Biomater 94 (2019) 392–409.
[61] F. Kito, et al., Establishment and characterization of novel patient-derived osteosarcoma xenograft and cell line, In Vitro Cell Dev Biol Anim 54 (7) (2018) 528–536.
[62] J.K. Anninga, et al., Chemotherapeutic adjuvant treatment for osteosarcoma: where do we stand? Eur J Cancer 47 (16) (2011) 2431–2445.
[63] N.M. Marina, et al., Comparison of MAPIE versus MAP in patients with a poor response to preoperative chemotherapy for newly diagnosed high-grade osteosarcoma (EURAMOS-1): an open-label, international, randomised controlled trial, Lancet Oncol 17 (10) (2016) 1396–1408.
[64] S.S. Bielack, et al., Methotrexate, Doxorubicin, and Cisplatin (MAP) Plus Maintenance Pegylated Interferon Alfa-2b Versus MAP Alone in patients with resectable high-grade osteosarcoma and good histologic response to preoperative MAP: first results of the EURAMOS-1 good response randomized controlled trial, J Clin Oncol 33 (20) (2015) 2279–2287.
[65] X. Xiao, et al., Individualized chemotherapy for osteosarcoma and identification of gene mutations in osteosarcoma, Tumour Biol 36 (4) (2015) 2427–2435.
[66] P.A. Meyers, A.J. Chou, Muramyl tripeptide-phosphatidyl ethanolamine encapsulated in liposomes (L-MTP-PE) in the treatment of osteosarcoma, Adv Exp Med Biol 804 (2014) 307–321.
[67] P.A. Meyers, et al., Osteosarcoma: the addition of muramyl tripeptide to chemotherapy improves overall survival–a report from the Children's Oncology Group, J Clin Oncol 26 (4) (2008) 633–638.
[68] D.M. Lussier, et al., Enhanced T-cell immunity to osteosarcoma through antibody blockade of PD-1/PD-L1 interactions, J Immunother 38 (3) (2015) 96–106.
[69] P. Puerto-Camacho, et al., Preclinical efficacy of endoglin-targeting antibody-drug conjugates for the treatment of ewing sarcoma, Clin Cancer Res 25 (7) (2019) 2228–2240.
[70] T. Kiyuna, et al., Eribulin suppressed cisplatinum- and doxorubicin-resistant recurrent lung metastatic osteosarcoma in a patient-derived orthotopic xenograft mouse model, Anticancer Res 39 (9) (2019) 4775–4779.
[71] H.J. Park, et al., The PARP inhibitor olaparib potentiates the effect of the DNA damaging agent doxorubicin in osteosarcoma, J Exp Clin Cancer Res 37 (1) (2018) 107.
[72] N. Martinez-Velez, et al., The oncolytic adenovirus Δ24-RGD in combination with cisplatin exerts a potent anti-osteosarcoma activity, J Bone Miner Res 29 (10) (2014) 2287–2296.
[73] M. Kawano, et al., Anti-TGF-β antibody combined with dendritic cells produce antitumor effects in osteosarcoma, Clin Orthop Relat Res 470 (8) (2012) 2288–2294.

[74] W. Schwinger, et al., Feasibility of high-dose interleukin-2 in heavily pretreated pediatric cancer patients, Ann Oncol 16 (7) (2005) 1199–1206.
[75] C.S. Fuchs, et al., A phase 3 randomized, double-blind, placebo-controlled trial of ganitumab or placebo in combination with gemcitabine as first-line therapy for metastatic adenocarcinoma of the pancreas: the GAMMA trial, Ann Oncol 26 (5) (2015) 921–927.
[76] N. Himoudi, et al., Lack of T-cell responses following autologous tumour lysate pulsed dendritic cell vaccination, in patients with relapsed osteosarcoma, Clin Transl Oncol 14 (4) (2012) 271–279.